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ATM Mutations and Phenotypes in Ataxia-Telangiectasia Families in the British Isles: Expression of Mutant *ATM* and the Risk of Leukemia, Lymphoma, and Breast Cancer

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Summary

We report the spectrum of 59 *ATM* mutations observed in ataxia-telangiectasia (A-T) patients in the British Isles. Of 51 *ATM* mutations identified in families native to the British Isles, 11 were founder mutations, and 2 of these 11 conferred a milder clinical phenotype with respect to both cerebellar degeneration and cellular features. We report, in two A-T families, an *ATM* mutation (7271T→G) that may be associated with an increased risk of breast cancer in both homozygotes and heterozygotes (relative risk 12.7; $P = .0025$), although there is a less severe A-T phenotype in terms of the degree of cerebellar degeneration. This mutation (7271T→G) also allows expression of full-length ATM protein at a level comparable with that in unaffected individuals. In addition, we have studied 18 A-T patients, in 15 families, who developed leukemia, lymphoma, preleukemic T-cell proliferation, or Hodgkin lymphoma, mostly in childhood. A wide variety of *ATM* mutation types, including missense mutations and in-frame deletions, were seen in these patients. We also show that 25% of all A-T patients carried in-frame deletions or missense mutations, many of which were also associated with expression of mutant ATM protein.

Introduction

Ataxia-telangiectasia (A-T; MIM 208900 [http://www3.ncbi.nml.nih.gov:80/htbin-post/Omim/dispmim?208900]) is an autosomal recessive disorder characterized by a progressive cerebellar degeneration leading to truncal and limb ataxia, dysarthria, and abnormal eye movements. In addition, classically, there are oculocutaneous telangiectasia and developmental and endocrine abnormalities, including hypogonadism, immunodeficiency, increased radiosensitivity, and predisposition to malignancies, particularly lymphoid tumors (Sedgwick and Boder 1991). In the vast majority of cases the symptoms of cerebellar degeneration are seen in infancy and certainly by the time the child starts to walk, at age 12–18 mo. The cerebellar ataxia gradually progresses, and, by their teens, patients are unable to walk and are confined to a wheelchair. Approximately 10%–15% of all A-T patients develop a malignancy in childhood or early adulthood. The vast majority of tumors, including all the reported leukemias, are of lymphoid origin (Spector et al. 1982). Although A-T patients develop both B-cell and T-cell tumors, the proportion of these two types in these patients is quite different than the proportion in the non–A-T population, and it is possible that most of the lymphoid tumors observed in A-T patients are of T-cell origin (Spector et al. 1982; Taylor et al. 1996*b*).

The gene for A-T, *ATM* (GDB:593364 [http://gdbwww.gdb.org/]) has been cloned (Savitsky et al. 1995*a,* 1995*b;* Byrd et al. 1996). The deduced amino acid sequence of the *ATM* gene contains 3,056 residues and, at its carboxyl-terminal end, shows similarity to the catalytic domain of phosphatidylinositol-3 kinases. The PI-3 kinase motif is common to a group of proteins, including those of *Drosophila melanogaster mei41*, *Saccharomyces cerevisiae TOR1* and *TOR2* (and their human homologues FRAP and rRAFT), *TEL1, MEC1, Schizosaccharomyces pombe rad3,* and the DNA-dependent protein kinase catalytic subunit (Savitsky et al. 1995*a,* 1995*b*) that are involved in cell-cycle regulation, response to DNA damage, interlocus recombination, and control of telomere length. The exact function(s) of the ATM protein is still to be established, although there is some evidence that the ATM protein is involved in meiotic recombination (Keegan et al. 1996) and, there-

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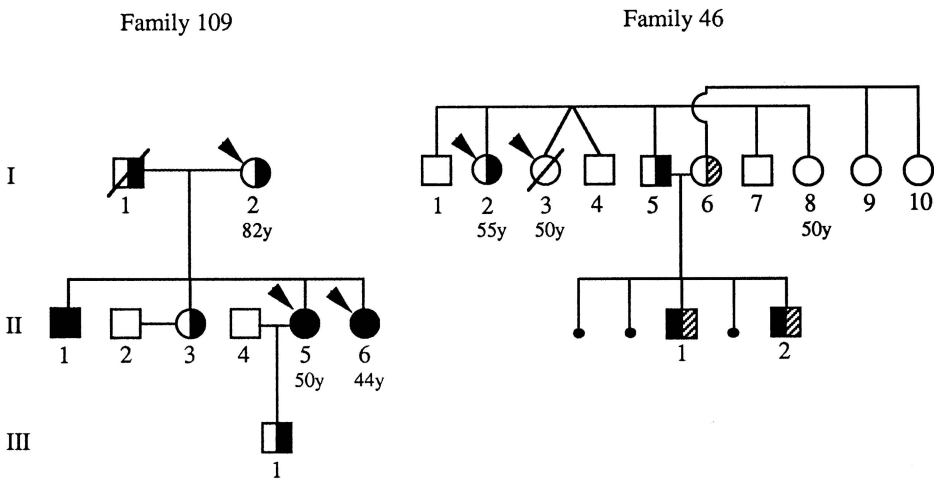

Figure 1 Pedigrees of families 46 and 109. Arrows indicate family members with breast cancer, whose ages are also given. In family 109 the affected patient 109 II-5 has a son. In family 46, the two A-T patients 46 II-1 and 46 II-2 each carry a 7271T→G and 3910del7nt *ATM* mutation indicated by a filled in and hatched symbol respectively. There were 3 miscarriages in this family.

fore, is likely to be associated with certain types of DNA processing, including processing of damaged DNA. Mutations that truncate the ATM protein predominate in A-T patients with the classically severe form of the disorder (Byrd et al. 1996; Gilad et al. 1996; Telatar et al. 1996; Wright et al. 1996). Phenotypic variations have been observed in a number of patients with A-T. The variations are mainly in the degree of neurological deterioration, levels of immunodeficiency, levels of cellular radiosensitivity, and presence or absence of tumors (Spector et al. 1982; Sedgwick and Boder 1991; Taylor et al. 1996*b*). Both the age at onset of cerebellar degeneration and its rate of progress have been reported to be variable. Families have been reported with either unusual genetic features (Swift et al. 1986) or both unusual genetic and unusual clinical features (Ying and DeCoteau 1981). Both the occurrence of a particular type of lymphoid tumor in more than one sibling in a significant number of families and concordance for tumor type in these cases (Spector et al. 1982; Taylor et al. 1996*b*) suggest strongly that cancer predisposition may be greater for some A-T patients than for others. It is possible that this is due to the presence of particular mutations within the coding sequence of the *ATM* gene. There is, therefore, a need for elucidation of the underlying molecular mechanism of phenotypic heterogeneity.

Although phenotypically normal, A-T carriers may also be at a higher risk of developing breast cancer (Swift at al. 1986, 1991). If shown to be true, this observation may have wide implications, since the frequency of the A-T carriers varies between 0.5% and 1%, depending on the population (reviewed by Easton 1994). Here we identify and analyze a large number of mutations in A-T patients from the British Isles, in terms of these mutations' locations within *ATM*, their expression at the protein level, and their relationship both to different phenotypic features and, particularly, to the occurrence of lymphoid tumors and breast cancer.

Patients and Methods

Patients

Lymphoblastoid cell lines (LCLs) from 78 patients in 68 A-T families were analyzed for the presence of mutations within the *ATM* gene. Of 24 A-T patients in the British Isles who were known to us and who had a hematologic malignancy or preleukemic T-cell clonal proliferation, we have analyzed mutations in 18. Of these 18 patients, 2 adults developed T-cell prolymphocytic leukemia (T-PLL), and 1 adult developed B-cell centroblastic lymphoma (Taylor et al. 1996*b*). Eight children developed acute lymphoma or leukemia of T-cell origin, two children developed lymphoma of B-cell origin, one child developed Hodgkin disease, and one child developed acute myeloid leukemia. Three further patients were diagnosed with chronic clonal T-cell proliferations characteristic of the preleukemic phase of T-PLL.

Family 109 (*fig. 1*).—Three members of a sibship of four have long-standing ataxia. Their parents were first cousins and originated from Orkney, in the north of Scotland. The proband, her older sister (with A-T), and her mother had breast cancer. There was no other history of malignancy in the affecteds or their other relatives. The affecteds had minimal telangiectasia and no obvious increased tendency to infections, except for recurrent urinary-tract infections in the brother (II-1) of the proband.

109 II-6 (the proband), age 48 years, had had truncal ataxia and progressive dysarthria since her early 20s. A computed-tomography scan at age 33 years showed cerebellar degeneration, mainly in the midline. At age 34 years, the patient had to discontinue her employment in a knitwear factory. She had ulcerative proctosis at age 31 years and developed fibrocystic disease of the breast at age 42 years. Two years later she presented with an invasive ductal carcinoma of the right breast, which was treated with lumpectomy and conventionally fractionated postoperative radiotherapy. Seven weeks later a very severe reaction in skin and breast tissue occurred, and since then her breast has remained firm, hot, edematous, and covered with telangiectasia. At this point the diagnosis of A-T was considered for the first time. Four years later, at age 48 years, the patient developed intraductal carcinoma in situ in the contralateral breast, which has been treated with lumpectomy only. She had minimal telangiectasia (except on the right breast) and dysarthric but comprehensible speech and was of normal intelligence. Despite severe truncal ataxia she could still just walk, with support. Peripheral ataxia was evident but less severe, and she could still do embroidery. Oculomotor apraxia was marked, and her fundi were normal. Her muscle strength was normal, muscle tone was slightly reduced, and reflexes were reduced in arms and absent in legs. Her plantars were flexor on the right and equivocal on the left. Vibration sense was reduced in her legs, but sensation was otherwise normal.

109 II-1 (the proband's brother), age 61 years, had had, according to his mother, abnormal head movements since age 3 years. Ataxia was first recognized when he was age 9 years. His neurological course has been similar to his sister's but slightly more severe. Unlike his sister, he had spontaneous choreiform movements and loss of proprioception.

109 II-5 (the proband's sister), age 50 years, has had a neurological course similar to that of the proband. Menarche occurred at age 14 years, and she had normal periods, which stopped at age 46 years. After many years of trying to conceive, she gave birth, at age 37 years, to a normal son and has had no miscarriages or stillbirths. At age 50 years she developed invasive ductal carcinoma of the right breast.

Family 46 (*fig. 1*).—There are two affected sons, ages 16 and 28 years, whose ages at onset of ataxia were 8 and 4 years, respectively. The ataxia progressed slowly in both brothers, but the older brother has deteriorated recently. He was a clumsy child with poor handwriting and poor games performance. He was just able to stand with his feet apart and had complex nystagmus with very broken saccades but no frank oculomotor apraxia. He had peripheral neuropathy, loss of most of the tendon reflexes, diminished pain sensation to the ankles, and mild wasting and weakness of the muscles of the feet. However, with support he was still able to walk short distances. He had frequent ear and chest infections. His younger brother, age 16 years, could still walk unaided, although this was tiring, and he used an electric scooter for long distances. He showed discrete involuntary movements in the upper limbs, but he still took part in games, including soccer. Like his brother, he also had frequent infections. Both patients had typical oculocutaneous telangiectasia.

Two of the three sisters of the father (46 I-5) in this family had breast cancer, one at age 50 years and the other at age 55 years. The third sister was age 50 years. The mother (46 I-6) in this family had had three miscarriages and also has a sister who, at age 44 years, had a lumpectomy of unknown pathology.

Biochemical and Immunological Investigations of Family 109

Alpha-fetoprotein, carcinogenic embryonic antigen, glycosylated hemoglobins, and peripheral blood–lymphocyte profiles were all normal in the affected sibs. The immunoglobulins, including IgE, were normal in II-5 and II-6. II-1 had raised IgG levels and some kappa paraproteinemia, but results of bone marrow examination were normal.

Haplotype Analysis

Genotyping of A-T individuals and their families was performed for the following loci: D11S2000, D11S1817, D11S1343, D11S1787, D11S1819, K28, ACAT, GS193, D11S2179, D11S535, D11S1778, D11S1294, D11S2180, D11S1818, and D11S2178. Multiallelic short tandem repeats (STRs) were amplified by PCR. Primer sequences used for the amplification of STR polymorphisms were kindly provided by Y. Shiloh in the case of loci S1817, S1819, S2179, S1778, S2180, and S1818 or obtained from the Genome Data Base. In each case, one primer was end labeled with γ^{33}P-ATP, and the PCRs were performed for 35 cycles. The products were run on 8% denaturing gel, and the gels were then dried and exposed to x-ray film for 12–18 h. K28 is a biallelic polymorphism obtained from a cDNA derived by direct selection from the A-T regional cosmids. For this polymorphism, genotyping was performed by digesting the PCR product with *Taq*I and running an agarose gel to identify different alleles. GS193 is a biallelic polymorphism observed on a denaturing gel (N. J. Lench, personal communication).

Identification of Mutations

Mutation analysis of the *ATM* coding sequence was performed by RT-PCR and three complementary methods: restriction-endonuclease fingerprinting (REF) (Liu and Sommer 1995; Byrd et al. 1996), heteroduplex anal-

ysis (HD) (Byrd et al. 1996), and protein-truncation test (PTT) (McConville et al. 1996). RNA was prepared from LCLs of individual A-T patients, and first-strand cDNA synthesis was undertaken by use of a SuperScript preamplification system (Gibco BRL) with an oligo (dT) primer. For the detection of mutations causing splicing defects, both exon/intron boundaries of the relevant exons were amplified from genomic DNA and were sequenced.

Production of Antibodies and ATM Protein Analysis by Western Blotting and Immunoprecipitation

An anti-peptide antibody (NT1) was raised, in sheep, against an N-terminal epitope of the ATM protein (14–28 amino acids [aa]). In addition, two rabbit antibodies, FP14 and FP8, were raised against the N-terminal cloned portions of the *ATM* gene (288–524 aa and 992–1,144 aa, respectively). Total cell lysates were obtained from ~10^7 cells by sonication in 1 ml buffer (8 M urea, 150 mM β-mercaptoethanol, and 50 mM Tris/HCl pH 8), followed by 30 min centrifugation to remove insoluble cellular debris. Alternatively, nuclear extracts were prepared from individual cell lines by a micropreparation method and high-salt extraction, as described by Andrews and Faller (1991). Immunoprecipitation of the ATM protein with the NT1 antibody was performed in buffer containing 0.7 M NaCl, 1% NP40, and 20 mM Tris/HCl pH 7.2. Samples containing equivalent amounts of protein were electrophoresed on 6% SDS-polyacrylamide gels and were blotted onto nitrocellulose membranes. Western blotting and detection were performed by use of an ECL system (Amersham) with either FP14 or FP8, depending on the epitope retained in each patient.

Chromosomal Analysis of Nonirradiated and Irradiated Lymphocytes

Whole-blood cultures were incubated with phytohemagglutinin for 72 h and with colcemid for the last hour. Metaphase spreads were Giemsa banded, and 50 mitoses were analyzed fully. Chromosomal radiosensitivity in the A-T families was analyzed as described elsewhere (Taylor et al. 1986).

Results

ATM Mutations in A-T Patients in the British Isles

Fifty-nine different mutations were identified across the coding sequence of the *ATM* gene, in 68 analyzed A-T families resident in the British Isles (table 1), including 18 *ATM* mutations that we have previously published (Byrd et al. 1996; Lakin et al. 1996; McConville et al. 1996). Sixty of the families were "native" to the British Isles, four families had one parent from the British Isles, and four families were immigrant. With the exception of homozygotes in five families (three native to the British Isles and two immigrant), all patients were compound heterozygotes. Both mutations were found in 32 families, and one mutation was found in a further 36 families. Forty-two mutations (71%) were predicted to lead to the premature termination of the protein, eight mutations (14%) were in-frame deletions (six were exon-skipping mutations, and two were small deletions), and nine mutations (15%) were missense mutations predicted to cause exchange of one amino acid for another. Mutations were scattered across the whole coding sequence of the *ATM* gene, and 43 of the mutations in the British Isles were different than those published by others (Gilad et al. 1996; Telatar et al. 1996; Wright et al. 1996). Interestingly, the same mutation, IVS62+1G→A, resulted in two different splicing defects, 8672del115nt and 8787ins14nt, in different patients, similar to the effects of a COL7A1 gene mutation reported, but in a single patient, by Gardella et al. (1996).

Expression of ATM protein was investigated in LCLs from 74 A-T patients in the British Isles. None of the cell lines from 40 of the A-T patients, predicted to cause prematurely terminated proteins, showed detectable ATM protein (data not shown). Mutant ATM protein of apparently full length was detected in patients with either missense mutations or in-frame deletions. In addition to patients with the founder mutations (FMs), 5762ins137nt and 7636del9nt, who showed a previously reported variable expression of normal and mutant ATM protein, respectively (Lakin et al. 1996), patients from a further 17 families showed expression of mutant ATM, some of which is shown in figures 2 and 3*B*.

There were 51 mutations in 60 A-T families "native" to the British Isles, which probably represents approximately half of all the A-T patients. Eleven of the 51 mutations identified in more than one family were confirmed as FMs by the presence of a common haplotype within the families (table 2). Only patients with either FM7 or FM9 showed a clinical phenotype distinctly less severe in terms of cerebellar degeneration. One of these (FM7) was a splicing mutation (5762ins137nt) (McConville et al. 1996), whereas the other (FM9) was a missense mutation (7271T→G) associated with a milder phenotype and an increased frequency of breast cancer.

7271T→G Mutation, Milder Clinical and Cellular Phenotype, and Breast Cancer

Two families (46 and 109) shared FM9, a 7271T→G transversion (table 2 and figs. 1 and 3*A*), and a common haplotype. The 7271T→G mutation was predicted to produce a change in codon 2424, with replacement of valine by glycine. This point mutation was unlikely to represent a silent polymorphism, for several reasons. (1) In spite of a thorough search for the mutation, by use

Table 1
***ATM* Mutations in A-T Patients in the British Isles**

Patient(s)	cDNA		Protein		Exon(s)
	Change	Nucleotide(s)	Change	Codon	
5-3, 5-4, 28-3[a]	T→C	2	M→stop	1	4
39-3, 39-4	Δ4nt	136	Termination	46	5
27-3	Δ146nt	186	Termination	63	6
21-5	Δ166nt	497	Termination	166	8
28-3	ΔT	634-640	Termination	214	8
118-3	ins4nt	794-796	Termination	266	9
76-3	ΔT	822	Termination	275	9
91-3	C→T	875	P→L	292	9
12-3	ΔTG	1290	Termination	430	12
11-5	ΔC	1355	Termination	452	12
33-5, 73-3, 88-3[a]	ΔAG	1561(2)	Termination	522	12
57-3	ΔA	1782	Termination	595	13
102-3	Δ96nt (IVS14+2T→G)[b]	1803	Δ32 aa	602	14
95-3, 95-4, 111-2	Δ126nt (2250G→A)[b]	2125	Δ42 aa	709	16
17-5, 19-3, 92-3[a]	ins9nt	2249	Termination	752	16-17
72-3, 72-4, 79-3[a]	ΔCT	2284	Termination	762	17
37-5	A→G	2302	N→D	768	17
4-3, 10-3/4, 51-6, 50-3[a]	Δ200nt	2639	Termination	880	20
118-3	Δ83nt	2839	Termination	947	21
25-4	T→A	3008	L→Q	1001	22
15-3	Δ174nt (IVS25-12T→A)[bc]	3403	Δ58 aa	1135	26
1-3, 20-3, 54-4, 117-3[a]	ΔG	3802	Termination	1268	28
49-4	ΔT	3856	Termination	1286	28
120−3	Δ7nt	3904	Termination	1302	28
46-3/4, 90-3	Δ7nt	3910	Termination	1304	28
77-3	ΔT	4388	Termination	1463	31
85-3	A→C	5071	S→R	1691	36
19-3, 57-3	C→T	5228	T→I	1743	37
25-4	ΔT	5366	Termination	1789	38
1-3, 14-4, 38-3/4, 40-3/4, 44-4, 45-3, 52-4, 59-4, 62-3, 79-3[a]	ins137nt (IVS40-1050A→G)[b]	5762	Termination	1922	40/41
12-3	ΔA	5910	Termination	1971	41
26-3/4	ΔA	6056	Termination	2019	43
37-5	Δ103nt (IVS44+1G→A)[b]	6096	Termination	2033	44
5-3/4, 112-3[a]	insTT	6405	Termination	2136	46
59-4	ΔAG	6412/6414	Termination	2139	46
106-3	ΔAG	6916	Termination	2306	49
69-3	insT	6988	Termination	2330	50
93-5	insA	6997	Termination	2333	50
68-3	Δ38nt	7270	Termination	2424	51
46-3/4, 109-1/5/6[a]	T→G	7271	V→G	2424	51
72-3/4	Δ6nt	7279	Δ2 aa	2427	51
47-3, 75-3, 6-3/4, 21-5, 22-3/4/5, 26-3/4, 39-3/4, 51-6, 62-3, 94-3[a]	Δ159nt Δ9nt	7630 7636	Δ53 aa Δ3 aa	2544 2546	54 54
76-3	C→G	7660	H→D	2554	54
27-3	ΔGA	7829	Termination	2610	55
36-4	Δ5nt	7878	Termination	2629	55
77-3	Δ83	7928	Termination	2643	56
29-4	A→G	8003	E→G	2668	56
42-3, 42-4	Δ13nt	8004	Termination	2668	56
3-3	A→T	8098	K→stop	2700	57
9-3	Δ117nt (8264del5nt)[b]	8152	Δ39 aa	2718	58
10-3, 10-4	ΔAA	8206	Termination	2736	58
63-3	A→T	8266	Termination	2756	58
8-4	Δ150nt (IVS59+1del4nt)[b]	8269	Δ50 aa	2757	59
74-3	ΔA	8405	Termination	2803	59
31-5	insAA	8478	Termination	2826	60
88-3	T→G	8480	F→C	2827	60
70-3	Δ115nt (IVS62+1G→A)[bd]	8672	Termination	2891	62
40-3/4, 75-3, 95-3/4[a]	ins14nt (IVS62+1G→A)[bd]	8787	Termination	2930	62/63
30-3, 52-4	C→T	9139	R→stop	3047	65

[a] FMs associated with a common haplotype in patients.

[b] Genomic changes underlying splicing errors are represented according to the mutation nomenclature suggested by the Ad Hoc Committee on Mutation Nomenclature (1996).

[c] Deletion of exon 26 is caused by the mutation IVS25-12T→A and not by 3576G→A. The normal sequence at the site of this mutation is aaattt, as reported by Uziel et al. (1996).

[d] Mutation IVS62+1G→A results in two different splice defects, FM 8672Δ115nt and FM 8787ins14nt.

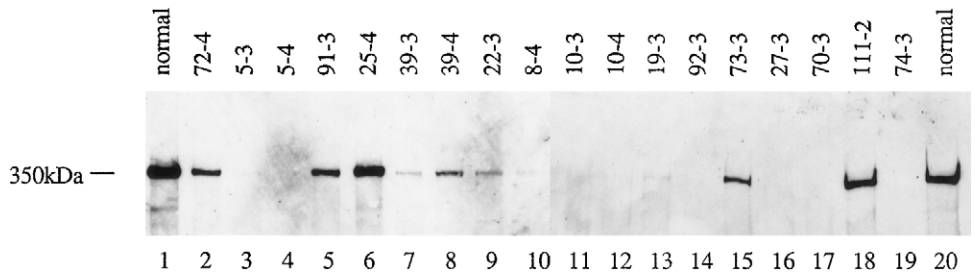

Figure 2 Western blot showing presence or absence of ATM expression in LCLs from A-T patients. Lanes 1 and 20, Extracts from two different normal LCLs showing a 350-kD band corresponding to the ATM protein of normal size. Lanes 2–19, Extracts from A-T LCLs with the patient designation given above the lanes (these are the same as given in table 4). Lanes 2–4, LCLs from patients with T-PLL or clonal proliferation. Lanes 5–12, LCLs from patients with T-cell tumors. Lanes 13 and 14, LCLs from patients with T-cell proliferation. Lanes 15–17, LCLs from patients with B-cell tumors. Lane 18, Acute myeloid leukemia. Lane 19, LCL from a patient with Hodgkins lymphoma. Lanes 2, 5–10, 13, 15, and 18, Varying levels of ATM protein, particularly low in samples in lanes 10 and 13. Lanes 3, 4, 11, 12, 14, and 19, Absence of any protein.

of both REF and HD, this change was the only one observed in family 109. (2) It segregated with A-T in both families. (3) It was associated with breast cancer in these two families. (4) The change was predicted to lead to the replacement of a large hydrophobic amino acid by the small amino acid glycine, in a part of the protein conserved between man and mouse. (5) The presence of this mutation was associated, in both family 46 and family 109, with a mild clinical phenotype and lower radiosensitivity, compared with those in classic A-T.

Family 109, in which the mutation was present in the homozygous state, contained the oldest patients in the British Isles who had demonstrable A-T—including one patient in his 7th decade, possibly the oldest A-T patient reported. This family also showed one further remarkable feature—one of the affected daughters had a son.

In family 46, the two affected brothers were compound heterozygotes for the *ATM* gene. In addition to the 7271T→G transversion, the second *ATM* mutation in this family was 3910del7nt. This mutation was predicted to cause premature termination of the ATM protein, but no truncated ATM protein was detected.

In both family 46 and family 109 a high frequency of t(7;14)-translocation chromosomes, typical of A-T, was observed in 72-h blood cultures derived from affected individuals (data not shown). In family 46 the level of x-ray–induced chromosome damage in the two affected boys was approximately two to three times greater than normal, and in family 109 it was approximately five to six times greater than normal (table 3), suggesting that lymphocytes from two sibs in family 46 were less radiosensitive than those from A-T siblings in family 109. The levels of induced damage in affected patients in these families were not as high as those seen in classic A-T patients, who show 10 times the normal level of induced damage (Taylor et al. 1996*a*).

Western blot analysis using ATM-specific antibodies revealed the presence of full-length ATM protein in the five affected individuals (fig. 3*B*) from families 46 and 109. Remarkably, the level of mutated ATM protein in the affected siblings of family 109 was similar to the level of ATM in both the carrier mother and normal individuals, whereas the levels of ATM in both A-T siblings heterozygous for mutation 7271T→G (46 II-1 and 46 II-2) were reduced compared with those in normal individuals (fig. 3*B*). This was consistent with the notion that it was the same mutated (with a single amino acid change) but stable 350-kD protein that was identified in all five affected individuals from families 46 and 109.

Both families sharing the FM 7271T→G had a history of familial breast cancer (fig. 1). In family 109 the carrier mother was age 82 years when she developed breast cancer, which may have been sporadic, but the two A-T daughters had breast cancers diagnosed, one at age 44 years and the other at age 50 years, and in one daughter the tumor was bilateral. The remaining daughter was an A-T gene carrier and, as yet, unaffected by breast cancer. Interestingly, two of three of the sisters of the paternal carrier of the 7271T→G mutation in family 46 have also had breast cancer, one at age 50 years and the other at age 55 years. We have confirmed that one sister with breast cancer was a carrier; but the second sister was deceased. The youngest sister without breast cancer was age 50 years and had not been tested for heterozygosity status.

The significance of the excess of breast cancer in these families is difficult to assess formally, since this excess may, to some extent, have led to the ascertainment of the families. In an attempt to correct for this, we have ignored the breast cancer cases occurring in the homozygotes and have considered only the risk of breast cancer to obligate heterozygotes or potential heterozygotes. This is appropriate, since, in our series, 7271T→G is the only ATM mutation in which breast cancer has occurred

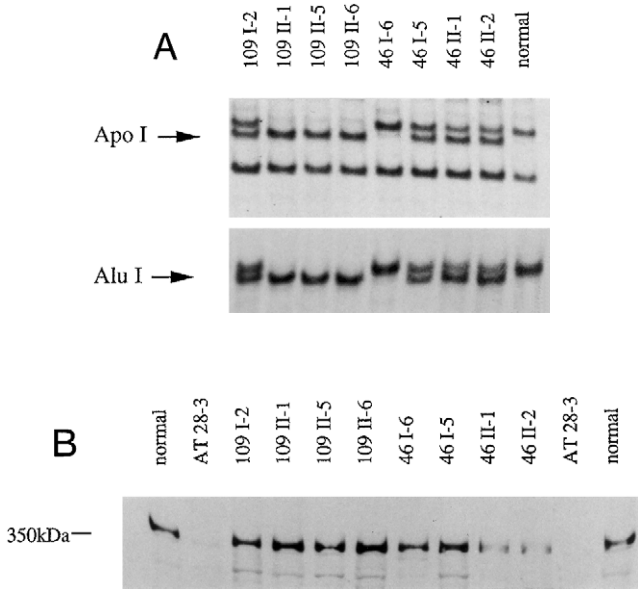

Figure 3 *A,* SSCP analysis of 185-bp RT-PCR fragment digested with restriction enzymes *Apo*I or *Alu*I for specific identification of the mutation common to individuals in families 109 and 46. The arrows point to a mobility shift in each digest, which is associated with the mutant allele. Lanes 1–4, Mother (109 I-2; heterozygous for the 7271T→G mutation) and the three affected siblings (109 II-1, 109 II-5, and 109 II-6, respectively, all of whom are homozygous for this mutation), from family 109. Lanes 5–8, Mother (46 I-6; not a carrier of the 7271T→G mutation), father (46 I-5; heterozygous for the 7271T→G mutation), and the two affected siblings (46 II-1 and 46 II-2; both of whom are heterozygous for the mutation). Lane 9, Normal control. *B,* Western blot analysis of LCLs derived from members of families 46 and 109, by use of ATM antibody FP8. Lanes 1 and 12, Extract of LCL from a normal individual, showing 350-kD band corresponding to the ATM protein of normal size. Lanes 2 and 11, Negative control from A-T patient 28-3, showing absence of ATM protein, as a result of mutations causing truncation of the protein from both alleles. Lanes 3-6, Mother (109 I-2; heterozygous for the 7271T→G mutation), showing presence of ATM protein of normal size, and the three affected siblings (109 II-1, 109 II-5, and 109 II-6, respectively; all of whom are homozygous for this mutation), also showing the normal size and levels of ATM protein. Lanes 7–10, Mother (46 I-6; not a carrier of the 7271T→G mutation), father (46 I-5; heterozygous for the 7271T→G mutation), and the two affected siblings (46 II-1 and 46 II-2; both of whom are heterozygous for the mutation), all showing presence of normal-size ATM protein but at lower levels in the affected siblings.

in more than one homozygote. The observed number of breast cancer cases has been compared with the number expected on the basis of the cumulative women-years at risk and the age-specific incidence rates for England and Wales during the period 1976–80. (These rates are not strictly appropriate for this comparison, since the follow-up is over a different period and since one of the families came from the Orkney Islands. However, incidence rates have not varied sufficiently to invalidate this approximation.) Two breast cancer cases occurred in confirmed carriers, and one further case occurred in a woman at 50% risk. In comparison, the expected numbers of breast cancer cases, based on age-specific incidence rates for England and Wales, would be .17 in obligate carriers and .31 in women at 50% risk, giving an estimated relative risk of 12.7 (95% confidence interval [CI] = 3.53–45.9), based on a weighted average of the observed and expected numbers weighted by the carrier probability and a significance level of .0025. For comparison, the relative risk of breast cancer in the homozygotes would be 74.1 (95% CI = 9.0–270.4).

ATM Mutations in A-T Patients with Leukemia and Lymphoma

Twenty different mutations were identified in 15 families (18 patients) with leukemia, lymphoma, or T-cell preleukemic clonal proliferations (table 4). Mutations were scattered across the *ATM* gene, suggesting that no single location within the *ATM* coding sequence was associated with the occurrence of lymphoid tumors. A spectrum of missense mutations, in-frame deletions, and null mutations was observed in patients with lymphoid tumors, with no particular *ATM* mutation attributable to any specific type of malignancy. However, more than half of the leukemia and lymphoma patients (in 8 of the 15 families) showed the presence of either missense mutations or in-frame deletions, all resulting in protein expression. In addition, we observed that both ATM null mutations and small in-frame deletions were associated with concordance for tumor type, in families in which two A-T patients developed a lymphoid tumor. For example, both affected siblings from family 10, compound heterozygous for the mutations 2639del200nt and 8206delAA, developed acute T-cell leukemia at a similar, young age. In contrast, each of two siblings in family 39 who had a 3-aa deletion and who developed acute T-cell lymphoma produced almost full-length ATM protein.

Discussion

Fifty-one different mutations were identified in 60 A-T families native to the British Isles. Although 71% of the mutations in the British Isles were predicted to lead to truncation of the protein, 29% were in-frame deletions and missense mutations and were associated with expression of some protein. These proportions are different from those in a previously published study (Gilad et al. 1996), which suggested that as many as 89% of mutations might inactivate the ATM protein by truncation. Since the present figures are derived from patients in a single geographic region, they are probably a better

Table 2

Founder *ATM* FMs and Haplotypes in Families Native to the British Isles

	No. of Mutations/Haplotypes										
D11S Marker	FM1 (2T→C), 2 Families	FM2 (1561delAG), 3 Families	FM3 (2249ins9nt), 3 Families	FM4 (2284delCT), 2 Families	FM5 (2639del200nt), 4 Families	FM6 (3802delG), 3 Families	FM7 (5762ins137nt), 10 families	FM8 (6405insTT), 2 Families	FM9 (7271T→G), 2 Families	FM10 (7636del9nt), 8 Families	FM11 (8787ins14nt), 3 Families
S2000											
S1817	2		14	14		14	4			2	
S1343	8	1	12	1		7	1	1		8	
S1787	6	1	2	1		4	3			6	
S1819	5	3	1	1	5	2	8	5		5	1
K28	1		2	1	1	2	1			2	1
ACAT	2		2	1	2	2	2			2	1
GS193	2	2	2	1	1	2	2		1	1	1
S2179	4	6	7	6	8	4	4		6	5	6
S535	2		1	2	1	1	2		1	1	1
S1778	1	1	1	4	11	1	2		7	12	5
S1294	3	6	2		2		1	4	1	3	4
S2180	3	4	4	2	4		4		5	4	4
S1818	3	1	2	3	1		1	4	11	10	
S2178	1	5	7	2	7		7		8	7	

Note.—The *ATM* gene is located between GS193 and S535.

Table 3

X-Ray–Induced Chromosome Damage in Lymphocytes from A-T Families 46 and 109, after Exposure to 1.0-Gray X-Rays at the G2 Phase of the Cell Cycle

	No. of Changes					
	Chromatid			Chromosome		
Individual (No. of Cells)	Gaps	Breaks	Interchanges	Fragments	Gaps	Breaks
109 I-2 (heterozygous) (50)	7	2	0	0	0	0
109 II-1:						
Sample 1 (50)	62	8	5	0	0	0
Sample 2 (50)	37	8	0	3	0	0
109 II-3 (heterozygous) (50)	8	1	0	0	0	0
109 II-5:						
Sample 1 (50)	53	14	5	1	0	0
Sample 2 (50)	48	9	4	1	0	0
109 II-6:						
Sample 1 (50)	51	11	6	3	1	0
Sample 2 (50)	58	15	4	7	1	1
109 III-1 (heterozygous) (50)	8	4	0	0	0	0
46 II-1	23	6	0	0	0	0
46 II-2 (50)	24	1	1	0	0	0
Normal 1 (50)	4	0	0	0	0	0
Normal 2 (50)	7	0	1	0	0	0

reflection of the frequency of mutation types. In addition, some of these "expressed" mutations may be associated with an increased risk of development of tumors, particularly breast cancer.

Clearly, there is clinical heterogeneity among A-T patients, particularly in terms of the severity of the cerebellar features, the level of immunodeficiency, longevity, the predisposition to breast cancer, and in the age at onset and the type of leukemia and lymphoma that these patients develop. We believed that a study of both the genotypes and phenotypes of a group of A-T patients might help our understanding of the causes of the clinical variations observed between patients. From this point of view, two families, 46 and 109, were particularly interesting. Both families carried the same 7271T→G mutation, which was localized several hundred base pairs upstream of the PI-3 kinase domain, and both showed a milder phenotype, in terms of cerebellar degeneration, than was seen in classic A-T patients, suggesting the presence of residual function in the mutant ATM protein. However, even between these two families there were clearcut differences in the phenotypes of the A-T patients.

Compared with family 46, family 109, homozygous for the 7271T→G ATM mutation, showed a milder phenotype, even in terms of longevity and fertility. Impaired fertility has been described as one of the major features of A-T (Sedgwick and Boder 1991); and this is the first example of fertility in an A-T homozygote. From studies in *atm* −/− mice, it is clear that the ATM protein is normally associated with meiotic chromosomes (Keegan et al. 1996; Xu et al. 1996) and may play a direct role in normal synapsis during meiosis. If this is also the case for A-T patients, then the high level of mutant ATM protein observed in family 109 may prevent the meiotic disruption, predicted from animal work, and may result in retention of fertility.

If the milder mutation alone determines the phenotype, then family 46, heterozygous for the 7271T→G mutation, might be expected to show a phenotype as mild as that in family 109. Since this was clearly not the case, another possibility is that the variation, in mildness of the clinical phenotype, between patients in families 46 and 109 was a consequence of the presence of the 7271T→G mutation in the heterozygous and homozygous states, respectively, and of the nature of the second mutation in family 46. The second mutation in family 46 was predicted to cause premature termination of the protein, but no truncated ATM was identified. It is likely, therefore, that the higher level of mutant protein expressed in family 109 contributed to the milder clinical phenotype, compared with that in family 46. This "dose response" effect would be consistent with the notion that there was residual function of this mutant ATM protein. A milder A-T phenotype in some patients therefore may result either from the presence, within the cell, of mutant ATM with residual function, in which the degree of mildness depends on the level of mutant ATM, or from the presence of a low amount of normal, intact protein (McConville et al. 1996).

Both female homozygotes and a heterozygote for the 7271T→G mutation in family 109, as well as female heterozygotes in family 46, showed an increased risk of breast cancer. Previous epidemiological studies (Swift et

Table 4

***ATM* Mutations in Patients with Leukemia and Lymphoma, and Their Expression at the Protein Level**

Type of Tumor, Patient(s)	Mutation	Putative Consequence for Protein	ATM Protein
T-PLL:			
5-3 and 5-4:[a]			
Allele 1	2T→C	Abolished translation	−
Allele 2	6405insTT	Termination after 2,153 aa	
72-4:			
Allele 1	2284delCT	Termination after 762 aa	+
Allele 2	7279del6nt	In-frame deletion of 2 aa	+
T-ALL:			
10-3 and 10-4:			
Allele 1	2639del200nt	Termination after 901 aa	−
Allele 2	8206delAA	Termination after 2,747 aa	
22-3:			
Allele 1	7636del9nt	In-frame deletion of 3 aa	+
Allele 2	Not determined		
8-4:			
Allele 1	8269del150nt	In-frame deletion of 50 aa	+
Allele 2	Not determined		
25-4:			
Allele 1	5366delT	Termination after 1,792 aa	
Allele 2	3008T→A	Missense mutation	+
T-cell lymphoma:			
91-3:			
Allele 1	875C→T	Missense mutation	+
Allele 2	Not determined		
39-3 and 39-4:			
Allele 1	136del4nt	Termination after 54 aa	
Allele 2	7636del9nt	In-frame deletion of 3 aa	+
Preleukemic T-cell proliferation:			
19-3:			
Allele 1	2249ins9nt	Termination after 753 aa	
Allele 2	5228C→T	Missense mutation	+
92-3:			
Allele 1	2249ins9nt	Termination after 753 aa	
Allele 2	2249ins9nt	Termination after 753 aa	−
B-cell lymphoma:			
70-3:			
Allele 1	8672del115nt	Termination after 2,899 aa	−
Allele 2	Not determined		
27-3:			
Allele 1	186del146nt	Termination after 65 aa	
Allele 2	7829delGA	Termination after 2,611 aa	−
73-3:			
Allele 1	1561delAG	Termination after 536 aa	
Allele 2	Not determined		+
AML:			
111-2:			
Allele 1	2125del126nt	In-frame deletion of 42 aa	+
Allele 2	Not determined		
Hodgkin lymphoma:			
74-3:			
Allele 1	8405delA	Termination after 2,805 aa	
Allele 2	8405delA	Termination after 2,805 aa	−

[a] Patient 5-4 shows T-cell clonal expansion but has not yet developed T-PLL.

al. 1991) have suggested a high risk of development of breast cancer in A-T heterozygotes relative to noncarriers. In a recent study, however (FitzGerald et al. 1997), *ATM* mutations were found to be present in only 2 of 401 women with early onset of breast cancer, compared with 2 of 202 controls. Although the existence of a breast cancer risk in A-T heterozygotes remains controversial, our observations on families 46 and 109 strengthen the evidence for an increased breast cancer risk in A-T gene carriers, as recently has been suggested by Athma et al. (1996). Our observation also raises the possibility that the risk may be higher for certain mutations and, therefore, that involvement of *ATM* gene mutations in breast cancer may be very significant in particular families.

Leukemia and lymphoma in A-T homozygotes in the British Isles are associated with a variety of *ATM* mutation types. It appears that the presence of two null alleles, with the consequent loss of all ATM protein expression, can result in a particular form of leukemia or lymphoma in one family, in a different form and at a markedly different age in another family, and, in the majority of A-T patients, in A-T without the tumor phenotype. Therefore, concordance for tumor type within families cannot be explained by the presence of null mutations, which are associated with the absence of ATM protein. Possible explanations for this difference between families with no expression of ATM include the influence of modifying genes, the differential expression of redundant genes, and/or environmental factors that may affect predisposition to the leukemia or lymphoma phenotype. When mutant ATM is expressed, as in the case of missense mutations and in-frame deletions, it is possible that heterogeneity of tumor phenotype may be attributable to the different properties of the various mutant proteins. Although we observed missense mutations and in-frame deletions expressed at the protein level in ~50% of A-T patients with various lymphoid tumors, Vorechovsky et al. (1997) recently have reported a clustering of *ATM* missense mutations in non–A-T patients with T-PLL, emphasizing the possible importance of *ATM* missense mutations in T-PLL development.

In summary, from our observations, it appears that specific mutant ATM protein can mitigate the neurological aspects of the clinical phenotype, as well as cellular features measured in vitro, but, at the same time, may promote a tumor phenotype. Other expressed in-frame deletions and missense mutations appear to contribute to the development of tumors, without the same mitigation of other aspects of the phenotype. In some A-T families, however, in which there is no ATM expression, the relationship of *ATM* mutations to tumor development appears to be more complex, and factors additional to the *ATM* gene may be involved.

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